#9 Priority Paper

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: **Indu J. ISAACS**

Title: ***GLP-2 Formulations***

Appl. No.: **09/750,022**

Filing Date: **December 29, 2000**

Examiner: **C. Kam**

Art Unit: **1653**

CLAIM FOR CONVENTION PRIORITY

Commissioner for Patents
Washington, D.C. 20231

Sir:

The benefit of the filing date of the following prior foreign application filed in the following foreign country is hereby requested, and the right of priority provided in 35 U.S.C. 119 is hereby claimed.

In support of this claim, filed herewith is a certified copy of said original foreign application:

- United Kingdom Patent Application No. UK Prov. 9930882.7, filed December 20, 1999.

Respectfully submitted,

Date June 10, 2002 (Monday)

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1/77

Request for Grant of a Patent

The Patent Office
Cardiff Road
NEWPORT NP9 1RH

1. Your re... 9930882.7 P46555

2. Patent Application num... *(the Patent Office will fill in this part)* -5 JAN 2000

3. Full name, address and postcode of the or of each Applicant *(underline all surnames)*

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Patent Office ADP Number *(if you know it)*

078...

If the applicant is a corporate body, give the country/state of its incorporation

Canada

4. Title of the Invention

GLP-2 Formulations

5. Name of your Agent *(if you have one)*

URQUHART-DYKES & LORD

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Patents ADP Number *(if you know it)*

1644005

6. If you are declaring priority from one or more earlier Patent Applications, give the country and the date of filing of the or of each of these earlier Applications and *(if you know it)* the or each

Country	*Priority application No. (if you know it)*	*Date of Filing (Day/month/year)*

7. If this Application is divided or otherwise derived from an earlier UK Application, give the Number and the Filing Date of the earlier Application

Number of earlier application	*Date of Filing (Day/month/year)*

8. Is a Statement of Inventorship and of Right to Grant of a Patent required in support of this request ?
(Answer 'Yes' if:

a) any Applicant named in part 3 is not an inventor, or
b) there is an inventor who is not named as an Applicant, or
c) any named Applicant is a corporate body.)

Yes

Patents Form 1/77

9. Enter the number of sheets for any of the following items you are filing with this form. Do not count copies of the same document

Continuation sheet of this Form	0
Description	10
Claim(s)	1
Abstract	0
Drawing(s)	6 + 6

10. If you are also filing any of the following state how many against each item

Priority documents	0
Translations of priority documents	0
Statement of Inventorship and Right to Grant a Patent *(Patents Form 7/77)*	0
Request for Preliminary Examination *(Patents Form 9/77)*	0
Request for Substantive Examination *(Patents Form 10/77)*	0
Any other documents *(please specify)*	0

11. I/We request the grant of a Patent on the basis of this Application

Signature Date

URQUHART-DYKES & LORD 30 December 1999

12. Name and daytime telephone number of person to contact in the United Kingdom

Mr G M Davies
01792 474327

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GLP-2 Formulations

Field of Invention

The present invention provides formulations for GLP-2 peptides and analogs thereof, particularly this invention relates to GLP-2 peptide formulations and GLP-2 analog formulations with improved storage stability.

Background

Administration of therapeutic peptides requires formulations of peptides which will remain stable. Generally parenteral administration is used with peptides because of their size. Peptides can be particularly difficult to formulate because of the tendency of peptides to breakdown over time.

GLP-2 is a 33 amino acid peptide, which is considered to have therapeutic applications in the treatment of diseases of the gastrointestinal tract. In particular, it has been determined that GLP-2 and analogs thereof act as trophic agents to promote growth of intestinal tissue and for enhancing the functioning of the upper gastrointestinal tract (see USP 5,834,428; USP 5,789,379; USP 5,990,077; WO 98/52600)

Commercial exploitation of GLP-2 requires the development of a formulation that is acceptable in terms of storage stability and ease of preparation. Because it is a protein and thus far more labile than the traditionally small molecular weight drugs, the formulating of GLP-2 presents challenges not commonly encountered by the pharmaceutical industry. For example, oxidation can occur at the methionine residue at position 10. Furthermore, GLP-2 may also be adsorbed to surfaces to form aggregates and/or precipitate.

Hence, it is desirable to provide a pharmaceutically useful and stable GLP-2 formulation or GLP-2 analog formulation.

Summary of the Invention

The present invention provides stable formulations of GLP-2 peptides and analogs thereof.

It has been discovered that relatively high concentrations of GLP-2 or analog thereof can be used in pharmaceutically acceptable formulations. Moreover, it has been discovered that a pH of greater than 5.5, more preferably greater than 6, even more preferably greater than 7, and most preferably approximately 7.3-7.4, is suitable for a stable formulation. It has also been discovered that the peptide undergoes a phase transition between 40-55°C depending upon the salt concentration and becomes hydrophobic in the presence of salt. It has also been discovered that Tween-80, salt and arginine are not appropriate materials for producing a stable formulation for GLP-2 peptides and analogs thereof.

According to one aspect of the present invention, there is provided a formulation, which provides for solubility for a medically useful amount, and preferably up to 10 mg/ml of GLP-2 peptide/analog, comprising a phosphate buffer to maintain the pH at a pharmaceutically acceptable level and above 5.5, a stabilizing amino acid, particularly L-Histidine, and Mannitol.

In an embodiment of the present invention, the formulation for the GLP-2 peptide, or analog thereof, particularly the h[Gly2]GLP-2, is a lyophilized formulation which comprises in the reconstituted product: phosphate buffer in an amount necessary to maintain the pH of the reconstituted product above 7, preferably to maintain a pH of approximately 7.3-7.4; 0.5-1% (w/v) L-Histidine; 2-5% (w/v) Mannitol, preferably 2.5-3.5% Mannitol, and most preferably approximately 3% Mannitol; and 0.1- 10% (w/v) GLP-2 peptide or analog thereof.

In a more preferred embodiment of the invention, a h[Gly2]GLP-2 lyophilized formulation is provided comprising in the reconstituted product: approximately 7-10 mg/ml of h[Gly2]GLP-2, preferably approximately 10 mg/ml; a phosphate buffer in an amount necessary to maintain the pH at approximately 7.3-7.4; approximately 0.5-1% (w/v) L-Histidine; and approximately 3% (w/v) Mannitol.

In another aspect of the present invention there is provided a process for making the lyophilized formulation of a GLP-2 peptide, or analog thereof, comprising the following steps:

(a) preparing the formulation of the GLP-2 or analog thereof comprising a phosphate buffer, L-Histidine, and Mannitol;
(b) freezing the formulation to –40°C;
(c) primary drying at -20°C; and
(d) secondary drying at +20°C.

In a preferred embodiment the formulation used in the lyophilization process comprises: the h[Gly2]GLP-2 analog; 35 mM Phosphate buffer to maintain the reconstituted product at a pH of approximately 7.3-7.4; 0.5-1% L-Histidine (w/v of reconstituted product); and 3% Mannitol (w/v of reconstituted product).

According to another aspect of the present invention, there is provided a method for obtaining a GLP-2 formulation for parenteral administration, which comprises the step of reconstituting the lyophilized formulation of the GLP-2 peptide or GLP-2 analog.

There is further provided in accordance with the invention, a therapeutically useful kit comprising a sterile vial containing a freeze-dried preparation of the invention, a vehicle suitable for reconstitution thereof, preferably sterile water, and instructions for reconstitution and optionally instructions for administration. The kit may further comprise a device suitable for injection of the reconstituted preparation.

Reference to the Drawings

Figure 1 is a graph showing the effect of certain amino acid stabilizers on a formulation using a heat stress test.

Figure 2 is a graph showing the effect of histidine on a phosphate buffered formulation.

Figure 3 is a graph showing the screening of bulking agents analyzed by RP-HPLC at room temperature and 60 °C.

Figure 4 is a graph showing the screening of bulking agents analyzed by SE-HPLC.

Figure 5 is a graph showing the stability of FMD-IV liquid formulations stored at 4 °C.

Figure 6 is a graph showing the stability of FMD-IV lyophilized formulations at room temperature and 60 °C.

Description of the Invention

The invention relates to GLP-2 peptide and GLP-2 analog formulations that exhibit storage stability. In particular, the lyophilized formulations of the present invention exhibit storage stability of at least 3 months at an ambient temperature, and more preferably of at least one year at 4 °C. Storage stability is exhibited by minimal peptide degradation, preferably less than approximately 5% peptide degradation, more preferably less than approximately 3-4% peptide degradation, even more preferably less than approximately 1-2% peptide degradation. Peptide degradation can be measured using standard reverse phase HPLC techniques.

The naturally produced GLP-2 peptides are highly conserved peptides. Accordingly, GLP-2 peptides for use in the present invention include the various naturally produced forms of GLP-2, particularly mammalian (such as primate, rat, mouse, porcine, oxine, bovine, degu, hamster, and guinea pig) or vertebrate forms (such as trout and chicken), and more particularly the human form. Desirably but not essentially, the GLP-2 selected for use is of the same species as the subject identified for treatment.

GLP-2 analogs potentially useful in the present invention are those compounds that activate the GLP-2 receptor by binding to that receptor and then stimulating an intracellular second messenger system coupled to that receptor. In embodiments of the invention, the GLP-2 analogs act selectively at the GLP-2 receptor. Selectively-acting GLP-2 analogs are compounds that, in the context of a suitable GLP-2 receptor binding or functional assay, bind to the GLP-2

receptor with greater affinity, desirably at least an order of magnitude greater affinity, relative to different receptor types, such as the GLP-1 receptor. In other embodiments, the GLP-2 analogs bind to the GLP-2 receptor with an affinity at least equivalent to the affinity of GLP-2.

The GLP-2 receptor analogs can be identified by screening peptides against cells engineered genetically to produce the GLP-2 receptor. The GLP-2 receptor has been cloned, as reported by Munroe *et. al.* in Proc. Natl. Acad. Sci, 1999 Feb 16; 96(4):1569. Cells functionally incorporating the GLP-2 receptor, and their use to screen GLP-2 analogs, are also described in WO98/25955 published 18 June 1998.

In other embodiments of the invention, the GLP-2 analog is an analog of natural GLP-2 that incorporates one or more amino acid substitutions, additions, deletions or modifications. Agonist activity of human GLP-2 and rat GLP-2 is believed to require an intact N-terminus, but various deletions of up to several residues at the C-terminus are tolerated without loss of agonist activity. Substitutions are tolerated at sites outside regions conserved across the various GLP-2 species homologs. Similarly, substitutions are also tolerated at sites within regions conserved across GLP-2 species. In preferred embodiments, the amino acid substitutions are conservative substitutions, for instance in which one member of an amino acid class is substituted by another member, e.g., the substitution of alanine by glycine, the substitution of asparagine by glutamine, the substitution of methionine by leucine or isoleucine, and the like.

In a preferred embodiment, the GLP-2 analog is a GLP-2 analog that has been altered to confer resistance to degradation by endogenous enzymes, such as DPP-IV. Such analogs suitably incorporate a replacement of the alanine residue at position 2. In specific embodiments, the Ala2 residue is replaced by glycine or serine, or by other residues as described for instance in U.S. 5,789,379. In a most preferred embodiment, the GLP-2 receptor agonist is [Gly2]GLP-2. For use in treating humans, the GLP-2 analog is desirably but not essentially a human GLP-2 peptide or analog, particularly including the Gly2 analog of human GLP-2.

It was discovered that the h[Gly2]GLP-2 analog precipitated at a pH of less than 5.5 and that there were temperature profiles suggesting a heat-induced and salt-dependent transition

temperature of approximately 40°C. Based on pH solubility profiles, phosphate was optimal in providing buffering capacity. Furthermore, histidine in the phosphate buffer was found to stabilize effectively. In particular, arginine and lysine are not recommended stabilizers. A stabilizing amino acid is an amino acid, which will increase the length of time that the peptide will remain intact before degrading then would otherwise happen absent the amino acid.

The lyophilized formulations of the present invention are provided preferably in a powder form containing not more than approximately 2% water by weight, and preferably not more than approximately 1% water by weight.

The excipient incorporated in the preparation should when used in the formulation produce a non-crystalline amorphous cake. It was found that lactose, trehalose and maltose were sugars which did not stabilize the formulation as well as mannitol and sucrose. More particularly, mannitol was found to be the preferred excipient.

The buffering agent incorporated in the formulation of the present invention is selected from those capable of buffering the preparation to a pH within a physiologically acceptable range for administration to a patient. More particularly, it was found that the pH of the formulation should by greater then 5.5, more preferably greater than 6, even more preferably greater than 7, and most preferably approximately 7.3-7.4. Most preferably, the buffering agent is phosphate based, most preferably a 35 mM Phosphate buffer is used.

The formulations of the present invention incorporate a GLP-2 peptide or analog thereof in a medically effective amount, namely an amount which is useful either therapeutically or diagnostically and which can be pre-determined based on the type of GLP-2 peptide or analog therof which is selected and on the intended end-use of the preparation. Therapeutically useful amounts of GLP-2 include those unit dosage amounts useful in a regimen to treat a subject that would benefit from GLP-2 administration, as described more fully in U.S. Patents 5,834,428, 5,789,379, 5,990,077, 5,952,301 and WO 98/52600. In one application, the formulation maybe exploited for the treatment of gastrointestinal disease, particularly diseases or conditions of the intestine. Therapeutically useful amounts also include multi-dose amounts of GLP-2 from which

multiple unit doses of GLP-2 can be delivered to an intended subject. Diagnostically useful amounts of GLP-2 include those amounts useful as calibrant when assessing endogenous levels of GLP-2 or levels of GLP-2 drug in a subject, for instance as a prelude to GLP-2 therapy, or during the course of GLP-2 treatment. Medically useful amounts of GLP-2 thus can range widely from a few micrograms to many milligrams.

In an embodiment of the invention, the formulations subject to the freeze-drying process comprises: approximately 7 mg/mL –10mg/mL of GLP-2 peptide or an analog thereof; approximately 2-5% (w/v of the reconstituted product) of mannitol, preferrably 2.5-3.5%, most preferably 3%; approximately 0.5-1% (w/v of the reconstituted product) of an amino acid stabilizer, preferably L-Histidine; and a phosphate buffer in an amount capable of buffering the reconstituted product to a pH of greater than 7, preferably a pH of about 7.3-7.4.

The GLP-2/analog formulation of the present invention is preferably filled in individual vials to the desired volume and the vials are subject to the lyopholization process. The lyopholization process includes a temperature cycling process that is carefully controlled to ensure that drying proceeds uniformly and until there is less than approximately 2% of water and preferably no more than approximately 1% of water. A lyopholization process suitable for the present invention involves a freezing step and a two step drying process. In the freezing step, the formulation vials are cooled from ambient temperature to –1°C at 2°C/minute and held at –1°C for approximately 15 minutes and then cooled from –1°C to –40°C at 2°C/minute and held at –40°C for 4 hours. In the first drying cycle, the temperature is increased from –40°C to –20°C at 2°C/minute and held at –20°C for approximately 14 hours under a vacuum of 150 mT with a condenser temperature of – 80°C. In the second drying cycle, the vials are warmed from –20°C to +20°C at 2°C/minute and held at +20°C for approximately 14 hours at a vacuum of 150 mT and a condenser temperature of –80°C until there is less than approximately 2% of water and preferably no more than approximately 1% of water. The vials are then preferably stored at 4°C.

The present invention also provides a medically useful kit comprising at least one vial containing the lyophilized freeze-dried GLP-2 or GLP-2 analog formulation of the invention, a vial of sterile water for reconstitution and instructions directing reconstitution and optionally an injection device for administration. To use the kit, the user mixes the water with the formulation

vial, preferably by transferring the water to the formulation vial. The formulation of the present invention rapidly dissolves and is stable for at least approximately 12 hours and up to approximately 24 hours.

The following examples are illustrative of the present invention but should not be a limiting factor in interpreting the scope of the patent application.

Example 1: Formulation and Lyophilization

Initially, a base formulation buffer composed of 35 mM sodium phosphate, pH 7.4, is prepared in the following manner: purified water is added to a sterile, depyrogenated flask and then sodium heptahydrate is added, followed by monobasic sodium phosphate monohydrate. The buffer is mixed and the pH is checked to be 7.4±0.2. This base formulation buffer is used to dilute h[Gly2]GLP-2 liquid bulk drug substance to a specified concentration of 10 mg/ml. At this point, L-Histidine is added to a final concentration of 7.76 gm/L and mannitol is added to a final concentration of 30 gm/L. The preparation is carefully mixed before being passed through a 0.22μm filter into a sterile filling tank. From this tank, the preparation is aseptically filled (in 1ml aliquots) into 3cc sterile USP Type I glass vials, which are then partially capped with sterile rubber stoppers and placed into lyophilization trays before being loaded into the lyophilizer. The lyophilization cycle is commenced by pre-freezing the formulation to a temperature of -40±2°C for approximately 4 hours. Primary drying of the drug product is performed at a temperature of -20°C for 14-17 hours, followed by secondary drying at –20 to 20±2°C for approximately 14-20 hours until the percentage of water is less then 1%. At the end of the lyophilization cycle, the vials are purged with filtered nitrogen and the rubber stoppers are fully depressed into the vials. The stoppered vials are removed from the lyophilizer and permanently sealed with a crimped aluminum seal and capped with a polypropylene flip-off button.

Example 2: Screening of Amino Acid Stabilizers

The h[Glyt2]GLP-2 formulation was tested with several amino acids as set out below. Citrate was included for mechanistic reasons because it provides three negative charges. The drug concentration was 10 mg/ml and the pH was maintained between 7.1-7.5. The formulations

were lyophilized according to the lyophilization protocol of Example 1 and then stored at 40 °C for 14 days before being heated at 60 °C for 4 hours.

1. 10mM Phosphate, 10 mM Glu
2. 10mM Phosphate, 10mM Glu, 50 mM Arg
3. 10mM Phosphate, 10 mM Citrate
4. 10mM Phosphate, 10 mM Citrate, 50 mM Arg
5. 10mM Phosphate, 100 mM-Citrate
6. 10mM Phosphate, 100 mM Citrate, 50 mM Arg
7. 10mM Phosphate, 10 mM Ser
8. 10mM Phosphate, 10 mM Ser, 50 mM Arg
9. 10mM Phosphate, 10 mM Pro
10. 10mM Phosphate, 10 mM Pro, 50 mM Arg
11. 10mM Phosphate, 10 mM His
12. 10mM Phosphate, 10 mM His, 50 mM Arg
13. 10mM Phosphate, 10 mM Gly
14. 10mM Phosphate, 10 mM Gly, 50 mM Arg
15. 10 mM His, 10 mM Gly
16. 10 mM His, 10 mM Gly, 50 mM Arg

All of the formulations containing arginine precipitated upon heating and did not survive the screening process. Formulation 5 (100 mM citrate) and Formulation 15 (histidine and glycine) also precipitated. There was a similar stability showing for the phosphate buffered formulations and 10 mM concentrations of histidine, citrate, serine, proline, glycine and glutamate. (See Figure 1) Furthermore, as shown in Figure 2, when histidine is used as a stabilizer in the phosphate buffer it stabilizes the formulation when it is heat stressed for 4 fours at 60 °C.

Example 3: Screening Bulk Agents

The following formulations of 10 mg/ml of h[Glyt2]GLP-2 were lyophilized using a similar lyophilization process as described in Example 1 and were then reconstituted and heated to 60 °C – all the samples precipitated at this concentration. Accordingly, the test was repeated at 0.4 mg/ml of h[Glyt2]GLP-2. As shown in Figures 3 and 4, the reverse phase HPLC data showed that the mannitol samples had the least degradation and all three His concentrations

showed comparable stability and the SE- HPLC analysis showed that except for maltose and lactose the GLP-2 analog in all the formulations eluted as a single peak without aggregation Formulation 6 and 7 gave an additional high molecular weight peak with approximately 6% purity. However when these samples were heat stressed at 60 °C, the purity of high molecular weight aggregates increased to approximately 20% in formulation 6 and 7. Accordingly, mannitol and sucrose were acceptable candidates based on this analysis. However, when the following liquid and lyophilized formulations (collectively referred to as FMD-IV) containing h[Gly2]GLP-2 at 10mg/ml were tested, sucrose did not stabilize as well as mannitol.

1. 35 mM Phosphate, 50 mM Histidine, 3% Mannitol, pH 7.4
2. 35 mM Phosphate, 50 mM Histidine, 5% Sucrose, pH 7.4
3. 35 mM Phosphate, 25 mM Lysine, 3% Mannitol, pH 7.4
4. 35 mM Phosphate, 25 mM Lysine, 3% Mannitol, pH 7.4

The concentration of sucrose in 3. was increased to 5% in order to meet in physiological osmolarity. For the lyophilization formulations, the lyophilization cycle of Example 1 was followed. Except for formulation 1, all other liquid samples degrade over time suggesting that sucrose content and/or lysine destabilizes. In particular, data points were taken on days 0, 7, 14, 21, 28, 42 and 49. (See Figure 5) On the other hand, the lyophilized samples stored at room temperature and 40 °C remained stable. However, the peptide did degrade extensively in lyophilized formulations 2-4 when the samples were heat-stressed at 60 °C for 4 hours. (see Figure 6)

Claims

1. A GLP-2 peptide or GLP-2 analog formulation suitable for administration into a subject comprising:

(a) a medically useful amount of GLP-2 or GLP-2 analog;

(b) a phosphate buffer in an amount sufficient to adjust the pH of the formulation to a pharmaceutically acceptable level and above 5.5;

(c) a stabilizing amino acid; and

(d) mannitol.

2. A GLP-2 peptide or GLP-2 analog formulation according to claim 1 wherein such formulation is lyophilized and comprises in the reconstituted product:

(a) approximately 0.1 – 10 % of GLP-2 peptide or analog thereof;

(b) a phosphate buffer in an amount sufficient to adjust the pH of the formulation to above 7;

(c) approximately 0.5–1% L-Histidine; and

(d) approximately 3% mannitol.

3. A GLP-2 peptide or GLP-2 analog formulation according to claim 2 wherein the GLP-2 peptide or analog thereof is h[Gly2]GLP-2.

4. A GLP-2 peptide or GLP-2 analog formulation according to claim 3 wherein there is approximately 10 mg/ml of h[Gly2]GLP-2.

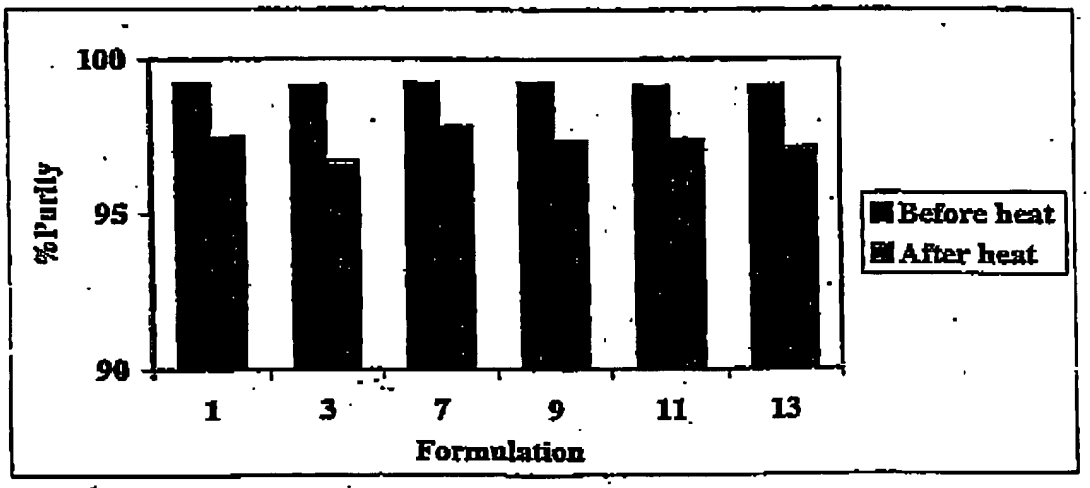

Fig. 1. Amino acids screening in buffers using heat stress.

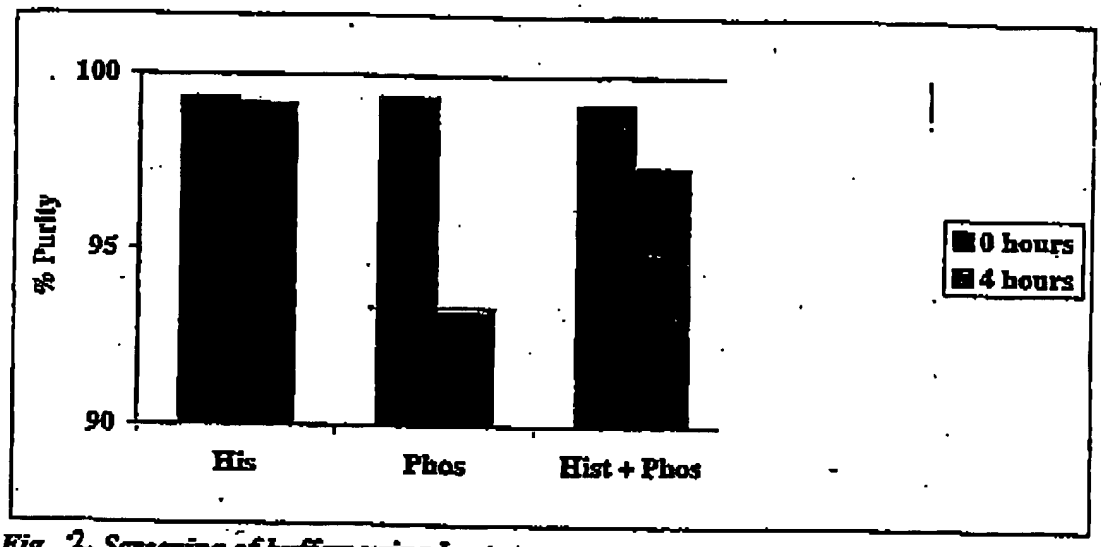

Fig. 2. Screening of buffers using heat stress.

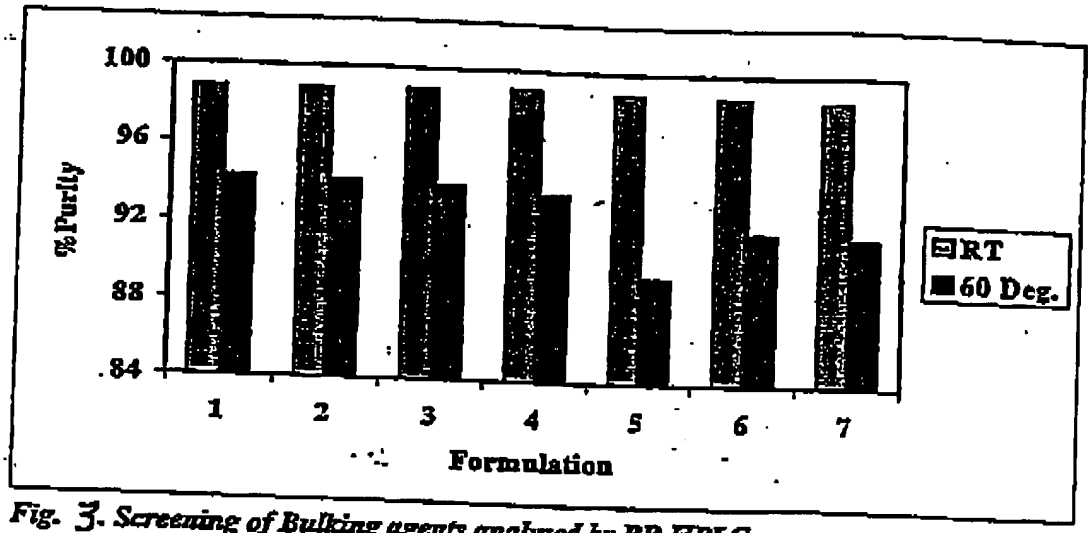

Fig. 3. Screening of Bulking agents analyzed by RP-HPLC.

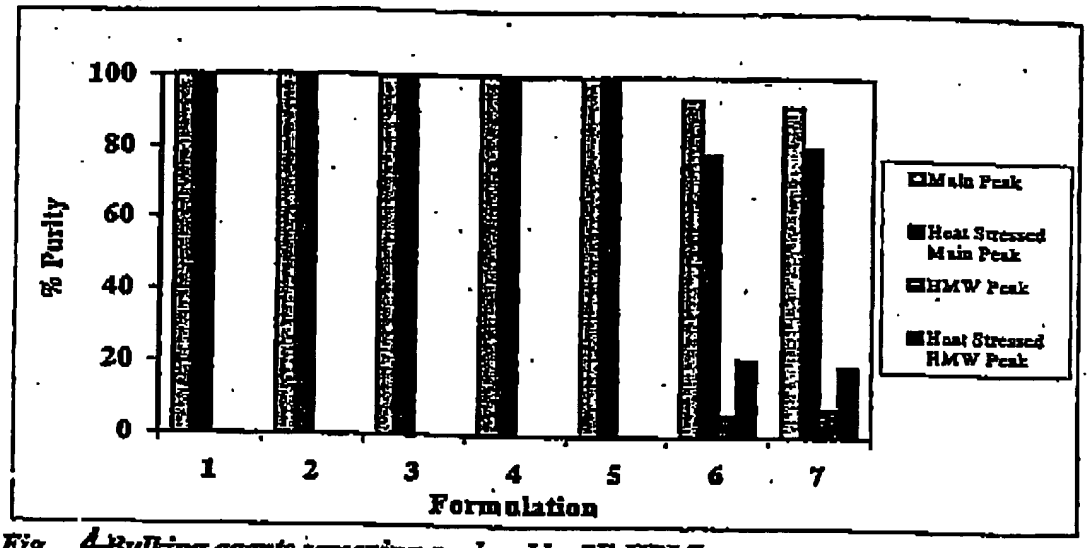

Fig. 4 Bulking agents screening analyzed by SE-HPLC.

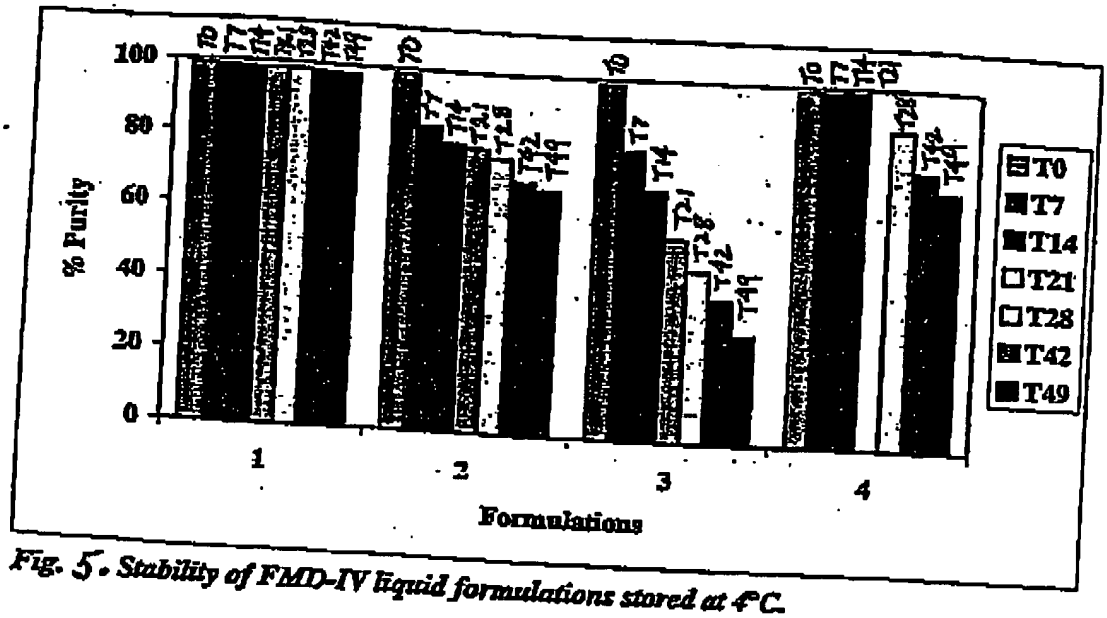

Fig. 5. Stability of FMD-IV liquid formulations stored at 4°C.

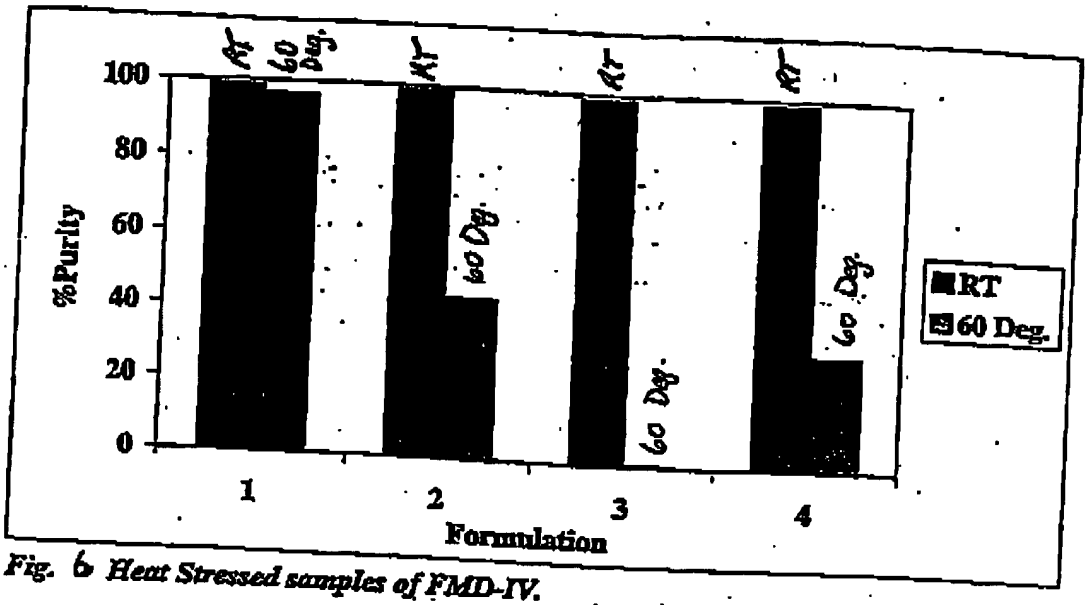

Fig. 6 Heat Stressed samples of FMD-IV.